Renal Lesions in Choline Deficiency in Rats

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DURING the course of experiments with rats fed on a vitamin A deficient diet deaths occurred from a hæmorrhagic degeneration of the kidneys. The changes found in the kidneys resembled those described in choline deficiency by Griffith and Wade (1939). In their experiments the hæmorrhagic renal lesion was always accompanied by marked fatty degeneration of the liver, while in our experiments the liver lesions were often minimal. Hartroft (1955) has recently reported fat embolization of the renal arterioles and capillaries in chronic choline deficiency and has supported the view that the fat is derived from the liver. The same author (1947 and 1948) described fatty infiltration of the proximal convoluted tubules in acute choline deficiency.

In our rats the relative absence of fatty change in the liver did not correspond with the accepted description of choline deficiency. Further experiments were carried out to determine if the renal changes were a manifestation of choline deficiency, and if there was any evidence of fat embolization in the kidney.

MATERIALS AND METHODS.

The original observation was made by one of the authors (M. G. McG.) during an experiment in which a group of 100 Wistar albino weanling rats was fed on a vitamin A deficient diet for the purpose of producing renal calculi (Higgins, 1933, 1935). The diet used was similar to Higgins' diet and was composed as follows :—

	per cent.
Caseinum soluble (B.P.C.)	18
Dextrin	65
Arachus oil	10
Dried brewer's yeast*	2
Salt mixture†	5

*Aluzyme, containing aneurin, nicotinic acid, riboflavine, pyridoxin, pantothenic acid and folic acid.

†NaCl, $MgSO_4$, NaH_2PO_4, KH_2PO_4, $CaHPO_4$, Fe citrate, Ca lactate.

Higgins did not report any deaths from choline deficiency kidney in his rats fed on this diet.

In the experiment in which the original observation was made 30 male rats died in a batch of 50, while only 4 of a batch of 50 females died (Experiment I in Table 1). Five further experiments were carried out, using 170 rats. In Experiment II rats aged 5 to 6 weeks were used and all the animals survived until they were killed after three weeks on the diet. The kidneys were found to be normal in all these rats. In the later four experiments weanling rats (aged 26 days) were

used, and in all of these experiments renal lesions were found in some of the animals. In Experiments II, III, and IV some of the rats were given supplements of choline chloride in their drinking-water, at the level of 35 mg. per rat per day in Experiment II, and 6 mg. per rat per day in Experiments III and IV and V. After it had been established that choline chloride completely prevented the lesions in the kidneys, Experiments V and VI were carried out to obtain material representative of the very early lesions and of the healing stage.

Post-mortem examination was carried out in all the animals except for a few which died during the night and were eaten by their litter mates. The animals which died and were eaten are presumed in Table 1 to have died from the renal condition as all deaths in the animals subjected to post-mortem examination were due to renal damage. The majority of the rats were killed by inhalation of ether in a closed chamber, a few being taken from each group from the ninth or tenth day onwards. All surviving rats were killed after three weeks. In Experiment VI animals were killed from the sixth day onwards. As a routine, blocks for histological examination were taken from the lobes of the liver and from the kidneys. In addition, in a small number of rats, blocks were taken from the lungs, heart, spleen, small and large intestine, skeletal muscle, brain, and spinal cord. The blocks were fixed in Helly's fixative, and in many rats duplicate blocks were fixed in 3 per cent. formaldehyde (7.5 per cent. formalin) for preparation of frozen sections for fat stains. Sections were stained with hæmotoxylin and eosin, and with Scharlach R for fat.

In some of the rats, bacteriological examination of the kidneys, spleen, and blood were carried out, but no organisms were seen in smears or grown in cultures.

Results.

On the fifth day on the diet affected rats began to appear thin. Deaths occurred from the seventh to the twelfth day. In all the experiments some of the rats appeared to escape, while animals in the same cage died. In the experiments in which rats were allowed to survive for three weeks some of the affected animals appeared to recover, although the diet was not changed and no choline was given. At post-mortem healing lesions were discovered in some rats which had not appeared to be ill. A summary of the experiments is given in Table 1.

Experiment I is the experiment in which the lesion of the kidney was first observed. None of these rats received supplements of choline. In Experiment II rats aged 5 to 6 weeks were used. Some of the rats were given choline, but neither these nor the rats which did not receive choline developed renal lesions. In Experiment III 26-day-old rats were used, some being given a supplement of choline. In this experiment renal degeneration occurred amongst the rats without the choline supplement, but not amongst those given choline. The absence of hæmorrhagic kidneys in Experiment II was thought to be due to a decreased susceptibility to choline deficiency in older rats. Griffith (1940) reported that there was a marked decrease in the incidence of renal lesions due to choline deficiency in rats aged 33 days and older. The rats used in Experiments IV, V, and VI were all aged 26 days. In Experiment IV choline was given to some of the rats and no

renal lesions occurred amongst these rats, although they were frequently present amongst those not given choline. The results of Experiments III and IV and V were taken as evidence that the renal lesion to be described later was a manifestation of choline deficiency.

Pathological Changes in the Kidney.

In the animals which died or appeared to be acutely ill the kidneys were found to be greatly enlarged, often to twice or three times the size of the kidneys of unaffected rats. The cortex was dark purple-red, contrasting with the grayish-red

TABLE 1.

Experiment	Age	Without Added Choline				With Added Choline			
		No. Male	No. Female	Renal Lesions No. Male	Renal Lesions No. Female	No. Male	No. Female	Renal Lesions No. Male	Renal Lesions No. Female
I	Weanling	50	50	30 (60%)	4 (8%)	0	0	0	0
II	5–6 weeks	10	10	0	0	6	0	0	0
III	26 days	7	6	3 (43%)	1 (17%)	4	3	0	0
IV	Weanling	13	12	9 (69%)	6 (50%)	12	13	0	0
V	Weanling	8	10	1 (12%)	7 (70%)	10	10	0	0
VI	Weanling	25	0	14 (56%)	0	0	0	0	0

of the medulla. The increase in size was due to swelling of the cortex. In animals which survived for twelve days or longer the kidneys were still enlarged, but less dark in colour. Later the cortex became yellowish brown in colour, with small purple-red patches here and there. These kidneys were larger than normal, but a considerable decrease in size had occurred when compared with kidneys of rats which had died in the acute stage. In a few of the animals surviving for three weeks the kidneys were found to be normal in size or slightly smaller than normal. The cortex was covered with dense white specks, which on the cut surface could be seen as streaks extending towards the cortico-medullary junction.

Microscopic Appearances.

The earliest lesion was the appearance of fine granules of stainable fat in the cells of the proximal convoluted tubules. This was already present at six days. A little later the fat granules were apparent in the loops of Henle and in the distal convoluted tubules. In the less severely affected cells the fat was situated at the base of the cells. The fat was present in kidneys in which the tubule cells appeared only slightly swollen when stained with hæmatoxylin and eosin. In the fully developed lesion the nuclei of many of the tubules had become pyknotic or had

disappeared and the cells had been replaced by amorphous eosinophilic debris. In some kidneys the necrosis involved the complete periphery of the cortex, but in others only small areas were affected. In the most severely affected kidneys the glomeruli were necrotic, but frequently these had escaped while their tubules were packed with fat or were necrotic. In some of the kidneys there was extreme congestion of the cortex, and hæmorrhage was present under the capsule, together with a little fibrin. Many of the tubules contained large eosinophilic casts which were most numerous in the region of the cortico-medullary junction. When stained for fat these casts contained little or no fat. The glomeruli were usually free from fat except when they had become necrotic, and there was seldom stainable fat in the blood vessels. The vascular system was normal except in areas where complete necrosis of the cortex had occurred.

In the healing stage proliferating fibroblasts appeared and calcium was deposited in necrotic tubule cells. Some of the casts also became calcified. A few mononuclear cells were sometimes present but infiltration with inflammatory cells was never marked. The necrotic cells gradually disappeared and in the oldest lesions there remained a wedge-shaped scar of fibrous tissue containing the calcified remains of a few tubules.

Pathological Changes in the Liver.

In the majority of the affected rats the liver appeared normal in colour, consistency, and size on naked-eye examination. In occasional rats it was slightly pale, but it was never enlarged. It was difficult to detect any macroscopic difference between the livers of the rats which had renal lesions and those in which the kidneys were necrotic. The livers were normal in the rats which had received choline.

Microscopic Appearances.

Microscopically the livers of most of the rats contained a little fat, whether they had received choline or not, but in many it was periportal in distribution. In rats without choline supplements to the diet sometimes the liver contained small amounts of centrilobular fat. The amount of this did not correspond to the presence or the severity of the renal lesions, some of the rats with histologically normal kidneys having considerably more fat than others in which the cortex was almost completely necrotic. The small amount of fat present in the livers of many rats with renal necrosis could not be explained by the rapid disappearance of liver fat during early healing, as it has been noted by Griffith (1941) and Christensen (1942) that the fatty infiltration of the liver remains during the recovery phase of the disease.

The fat in the livers and kidneys was not doubly refractile.

No abnormalities were detected in the lung, heart, spleen, intestine, skeletal muscle, brain or spinal cord in those rats in which they were examined.

Discussion.

The fully developed lesion in the kidneys of these rats bears a close resemblance to the choline deficiency kidney first described by Griffith and Wade (1939), and

also reported by Christensen (1942), Wachstein (1944), Baxter (1947), and Hartroft and Best (1947). It resembles Griffith's hæmorrhagic degeneration of the kidneys in that it can be prevented by choline. However, in Griffith's rats the liver showed marked fatty degeneration, and both he and other workers have reported that amounts of choline sufficient to prevent renal necrosis do not protect the liver, the liver being apparently more sensitive to choline deficiency than is the kidney. It was interesting that in the experiments described here the rats with the most severely affected kidneys often had relatively little fat in the liver, while some animals with fatty livers had normal kidneys. While the majority of reports of choline deficiency kidneys state that the liver was very fatty, Baxter (1947) reported that "most of his rats with hæmorrhagic kidneys had slightly to markedly fatty livers."

In these experiments the glomeruli were often undamaged when the tubules beneath the renal capsule had become necrotic, and only when extensive areas of the cortex had become necrotic were they involved. The primary lesion in choline deficiency kidney appears to be damage to the tubules. Dessau and Oleson (1947) also report that the glomeruli tend to escape in acute choline deficiency. However, the renal lesions in chronic choline deficiency involve the glomeruli (Hartroft, 1955), and the histological appearance is quite different from that of acute choline deficiency.

Griffith (1941) observed abundant fat droplets in the bases of the necrotic cells. Hartroft and Best (1947) reported the early appearance of fat droplets in the proximal convoluted tubules, increasing in size and number until congestion, hæmorrhage, and necrosis occurred. Hartroft (1948) suggested that the necrosis might be due to compression of the cortical capillary plexus by the swollen fat-filled cells of the proximal convoluted tubules which he called tubular obstruction of capillaries. In the experiments reported here congestion was not invariably a marked feature of the necrotic kidneys, although it occurred in many of them. This suggests that tubular obstruction of capillaries is unlikely to be the cause of the necrosis. Dessau and Oleson (1947) also favoured interference with the vascular outflow of the kidney as the cause of necrosis in choline deficiency.

The source of the fat in the cells of the renal tubules is not often considered. It would seem unlikely to have been carried to the cells in the blood stream, as the glomeruli are usually free of fat while the cells lining their tubules are full of it. The fact that the degree of fatty degeneration of the liver often does not parallel the renal changes would also tend to exclude the possibility of the renal fat arriving via the blood stream. It is possible that fat from the blood stream could have been filtered through damaged glomeruli and then have been reabsorbed by the tubules. However, the fat lies near the basement membrane of the tubules instead of near the lumen, as it would be expected to do if it had been absorbed from the glomerular filtrate. The casts, which are numerous, are not composed of fat, as they would have been if fat had escaped through damaged glomeruli. The most likely explanation of the fat in the renal tubules would appear to be a direct result of choline deficiency on the tubule cells, causing a phanerosis of fat already present in non-stainable form.

The diet used in these experiments was low in vitamin A content as well as in choline, but it was not completely absent from the casein preparation used. Other batches of animals on the same diet did not develop signs of vitamin A deficiency until after several months. The renal changes occurred within seven to twelve days, and it is unlikely that these were related to or modified by vitamin A deficiency.

Summary.

Degenerative lesions in the kidneys of rats fed on a vitamin A deficient, choline deficient diet are described. The earliest change was a fatty infiltration of the renal tubule cells, which progressed to necrosis. Glomerular involvement was often minimal and appeared to be secondary to tubular damage. There was no evidence that it was of vascular origin, and it would appear to be due to specific effect of choline deficiency on the tubular cells. Fatty infiltration of the liver occurred in some of the rats, but it frequently did not correspond in severity to the degree of renal damage.

We wish to thank Professor J. Henry Biggart for his advice in carrying out this work. Mr. J. Davidson kindly prepared the sections.

M. G. McGeown wishes to acknowledge the receipt of a grant from the Medical Research Council.

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REVIEW

A TEXT-BOOK OF MENTAL DEFICIENCY. By R. F. Tredgold, M.D., D.P.M., and K. Soddy, M.D., D.P.H. Ninth Edition. (Pp. xv + 480; plates 31. 40s.) London, Baillière, Tindall & Cox, 1956.

This text-book was first compiled by the late A. F. Tredgold and for many years was a standard text-book and one of the classics of the subject. The present work is in great part rewritten, but the late author's case histories and descriptions have been retained and give an interesting early century atmosphere to a book where the conflicting theories of modern psychology are presented and related to other aspects of mental deficiency. The practical and legal aspects of the problem also receive a balanced presentation.